

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

136

12/14/94

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Purpose: To try Ayauchi in balance & see in transformation whether we get better &.

L 1 = Tag 1 U (200  $\mu$ M each nucleotide) 1

L 4 = Tag 4 U " " 2

Tag: H 1 = " 1 U 200  $\mu$ M dA & rest 1 mM 3

H 4 = " 4 U " " 4

L-D 1 = Tag - DV 1 (200)

L-D 4 = " 4 " "

H-D 1 = " 1 (200 + 1 x 3)

H-D 4 = " 4 " "

Supernatants of each reaction pooled together, ethanol added after a phenol chloroform extraction.

Resuspended in 15  $\mu$ l reaction TE and rest with Aat II in NEB buffer overnight at 37°.

2  $\mu$ l of each run on gel to see the digestion is complete.

Even though Ayauchi said there is enough product in PCR & some of them didn't show up on the gel after all the purification steps.

Since there is not much time to gel purify the fragments whole reaction as such, was used in the ligation reaction.

2 10  $\mu$ l of it.

T Page No.

Witnessed &amp; Understood by me,

Date

Invented by

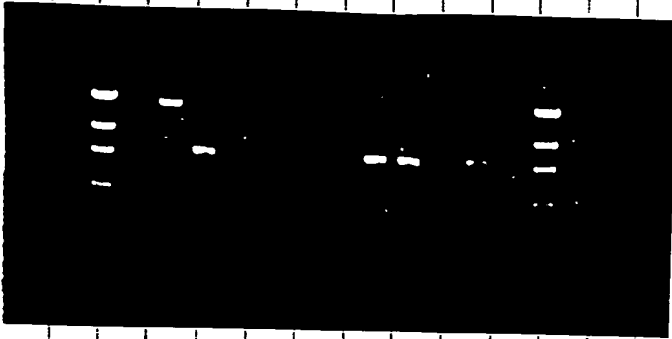
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Dr. Srinivasan

**ag No.\_\_\_\_**



Vector      Tag      Tag + Rx

Neutri	1 ml
insult	10 ml
ligase	1 ml
TE	
5x buffer	4 ml

200 ml at 25<sup>°</sup>, 3 hrs.

transformed all 10 (1-10 above) 8) Control insert minus per.

legation

- |     |        |        |
|-----|--------|--------|
| 1.  | Vector | only   |
| 2.  | Vector | + rest |
| 3.  | Targ   | 2 1    |
| 4.  |        | 1 1    |
| 5.  |        | 1 1    |
| 6.  |        | 1 1    |
| 7.  | T + 2v | 2 D 1  |
| 8.  |        | 2 D 4  |
| 9.  |        | HD 1   |
| 10. |        | HD 4   |

100 per 2  
Restripiclu  
don't  
knew  
exactly  
how much  
it is there

result is three

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~~sed & Understood by me,~~

Date \_\_\_\_\_

**Invented by**

**Date**

Recorded by

12/16/94

St. Blaasman